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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)		
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Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

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Date 02/24/2004

REGISTRATION NO. 54,594 *(if appropriate)*

TYPED or PRINTED NAME Jingxi Chu

Docket Number: 04UMC007

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Additional Page

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PROVISIONAL PATENT
File No. 04UMC007

ORGAN PRINTING USING SELF-ASSEMBLING MULTICELLULAR SYSTEMS

[0001] This invention is made by Gabor Forgacs, Karoly Jakab, Adrean Neagu, and Vladimir Mironov.

FIELD OF THE INVENTION

[0002] The present invention relates to the field of tissue engineering and organ assembly, and more particularly to organ printing with multicellular systems.

BACKGROUND OF THE INVENTION

[0003] Tissue engineering provides promising solutions to the growing demand for organ/tissue replacement therapies coupled with a chronically low supply of transplantable organs. For example, in the United States, over 80,000 people are on the national waiting list for organ transplants, and about seventeen will perish each day for lack of suitable organ replacements. To lessen and eventually solve the organ transplantation crisis, tissue engineers need to be able to build and grow transplantable human organs or organ substitutes in a laboratory, with high precision, on large scale, and fast.

[0004] A variety of methods and devices for tissue engineering have been attempted and developed with limited success but far from fulfilling the need. In particular, assembly of vascularized three-dimensional soft organs remains a big challenge. Existing tissue engineering technologies are based on seeding cells into biodegradable polymer scaffolds or gels, expanding and culturing them in bioreactors for several weeks, and finally implanting the resulting tissue into a patient's organ where the maturation of the new organ may or may not take place. Existing technologies have several disadvantages: 1) in their current forms, existing technologies do not work with large tissues that require a complex vascular network; 2) existing technologies assume that the scaffolds, often designed by computer modeling, would provide the desired shape and required stability for a target organ, which is not the case in most instances; 3) the seeding process in existing technologies is time consuming, and the seeded cells may not survive long enough to sufficiently proliferate; and 4) since the aim of existing technologies is mostly to produce only irregular tissue constructs, adaptation to rapid prototyping, i.e., fast automation, has not been attempted.

[0005] Therefore, what is needed is a new and improved technology that enables rapid and precise building of target organs. What is further needed is a method, combined with appropriate devices, capable of providing mechanically stable and maintainable three-dimensional organotypic structures.

SUMMARY OF THE INVENTION

[0006] The present invention, Organ Printing with Bio-ink, provides a new and improved method for tissue engineering and organ formation with high precision, stability and speed. The invention also discloses a new and improved building block in tissue engineering, Bio-ink particles, the making of such and the use thereof. The invention further teaches a new modeling method for the optimization of biodegradable polymers or gels in relation to Bio-ink particles used in a specific Organ Printing process.

[0007] In accordance with the teaching of the invention, instead of seeding isolated cells into pre-determined scaffolds as in the existing technologies, the inventive Organ Printing method employs certain cell aggregates, "Bio-ink" particles, as building blocks, to be deposited ("printed" or "implanted") directly on the surface of a stimuli-sensitive matrix made of biodegradable gels or polymers. The inventive method comprises three major sequential steps: (i) development of "blueprints" of a target organ (pre-processing), (ii) actual organ printing (processing), and (iii) organ conditioning and accelerated organ maturation (post-processing). Accordingly, the system carrying out the inventive method includes a control unit, a dispensing unit, and a maturation unit. The control unit comprises a computer that contains the information on the shape of a target organ and the properties of the matrix and provides instructions for the dispensing unit. The dispensing unit is in electronic communication with the control unit and comprises 1) a plurality of dispensers separately holding the Bio-ink particles and the pre-selected biodegradable gels or polymers and 2) a dispensing platform. The maturation unit is a bioreactor that assures the proper post-process handling of the resulting construct.

[0008] The inventive method represents a computer-aided layer-by-layer deposition process. First, a layer of biodegradable gels or polymers with pre-determined properties (e.g., density and composition) is dispensed onto the dispensing platform to form a "bio-paper," i.e., a solidifying layer of stimuli-sensitive matrix. Second, a layer of Bio-ink particles is deposited, according to a prescribed pattern, directly into the bio-paper. These two steps are then repeated until the desired construct is fully deposited. Subsequently, the Bio-ink particles are left in the maturation unit to fuse into the desired tissue or organ geometry. Finally, the bio-paper is eliminated via standard procedures.

[0009] The inventive Bio-ink particles are certain cell aggregates with prescribed shape and sizes. In particular, the present invention discloses that spherical or cylindrical cell aggregates are ideal as Bio-ink particles to be used as building blocks in the inventive Organ Printing technology. Using Bio-ink particles as building blocks instead of the isolated cells in existing technologies has numerous advantages. First, using Bio-ink particles can significantly reduce the processing (actual printing) time to achieve the desired structure. Reduced processing time enhances cell survival. Second, Bio-ink particles provide critical cell concentrations, which are difficult to achieve by other methods. Third, because Bio-ink particles may contain several cell types and a pre-built internal structure is easy to engineer, considerable time can be saved during post-process tissue and organ maturation. Finally, the mechanical hardship involved in the dispensing process is less damaging for Bio-ink particles than for individual cells.

[0010] The present invention further discloses a modeling method for the optimization of biodegradable polymers or gels in relation to Bio-ink particles used in a

specific Organ Printing process. According to the teachings of the invention, depending on the properties of the polymers or gels used, the Bio-ink particles either fuse into a three-dimensional structure of desired geometry in the post-processing step or the constituent cells disperse into the surrounding matrix. The inventive modeling method can aid the selection of biodegradable polymers or gels compatible to the Bio-ink particles by considering, among other factors, the aggregate-gel interfacial tension. The inventive modeling method also reveals that the three dimensional structure of desired geometry could be made to represent a metastable state of the cellular system, whose lifetime depends on the magnitude of cell-cell and cell-matrix interactions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig.1 is a schematic diagram of the Organ Printing system hardware according to the invention.

[0012] Fig. 2 is a schematic representation of the principle of Organ Printing technology according to the invention.

[0013] Fig. 3A shows modeling image of a particular metastable configuration, namely a toroid.

[0014] Fig. 3B shows a series of modeling images resulting from the fusion of individual aggregates.

[0015] Fig. 4 shows a series of fluorescence images of (living) cell aggregates fusion.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The present invention is a new Organ Printing method for tissue engineering. Organ Printing as defined by the teaching of this invention is a computer-aided, dispenser-based, three-dimensional tissue-engineering technology aimed at constructing functional organ modules and eventually entire organs. The inventive Organ Printing method comprises three major sequential steps: (i) development of "blueprints," (i.e. an electronic mold for the shape) of a target organ, also known as the pre-processing step, (ii) actual organ printing that dispenses biodegradable polymers or gels and Bio-ink particles in a prescribed manner to form a prescribed three dimensional construct, also known as the processing step, and followed by (iii) organ conditioning and accelerated organ maturation, also known as the post-processing step. Accordingly, the system accomplishing the inventive method comprises three major compartments, as shown in Fig. 1: the control unit [100], the dispensing unit [200], and the maturation unit [300].

[0017] The control unit [100] comprises a computer storing the anatomical "blueprints" (i.e. map) of target organs and the information on the desired biodegradable gels or polymers in relation to the Bio-ink particles used. The blueprint can be derived from digitized image reconstruction of a natural organ or tissue. Imaging data can be derived from various modalities including noninvasive scanning of the human body or a detailed 3D reconstruction of serial sections of specific organs. Optimal properties of the biodegradable gels or polymers in relation to the Bio-ink particles to be used for a specific application can be determined by the inventive modeling method (details disclosed below).

[0018] The dispensing unit [200], which is in electronic communication with the control unit [100], functions as a special purpose delivery device, capable of depositing Bio-ink particles and biodegradable gels or polymers, according to the instructions from the control unit [100]. The dispensing unit [200] comprises a plurality of dispensers [210] and at least one dispensing platform [250]. Some dispensers hold the pre-selected Bio-ink particles, while other dispensers hold the pre-selected gel or polymer. The dispensing platform [250] is a temperature-controlled substrate onto which the Bio-ink particles and gels or polymers are deposited. A variety of printing or dispensing devices can serve as a dispensing unit [200], such as, jet-based cell printers, cell dispensers or bio-plotters.

[0019] To achieve the desired organ structure, the dispensing unit [200] instructed by the control unit [100] deposits the biodegradable gels or polymers and the Bio-ink particles alternatively layer-by-layer. The resulting structure is then left in the maturation unit [300] at predetermined conditions to allow the Bio-ink particles to fuse into the desired tissue or organ. The maturation unit [300], depending on the complexity of the organ module, can comprise a simple incubator or a specifically designed bioreactor.

[0020] Fig. 2 shows a simplified schematic representation of the principle of the inventive method. First, the pre-selected gels or polymers are deposited to form a solidifying layer of "bio-paper" [400]. Second, the pre-selected Bio-ink particles are deposited or implanted into the solidifying bio-paper [400], in close apposition of each other, to form a dotted circle [410] or any other prescribed pattern. The preceding two

steps are sequentially repeated resulting in a closely associated but disconnected tube-like construct [420], which then is left to fuse into a complete tube of tissue [430].

[0021] The present invention teaches that Bio-ink particles upon deposition into a stimuli-sensitive matrix made of biodegradable polymers or gels have the ability to fuse into three-dimensional organ structures. The ability of cell aggregates to fuse is based on the concept of tissue fluidity, according to which embryonic tissues (or more generally, tissues composed of uniformly adhesive cells) in many respects can be considered as liquids. In particular, in suspension or on non-adhesive surfaces, various multicellular aggregates (comprising uniformly adhesive cells) round up into spherical shape similarly to liquid droplets. Therefore, closely placed aggregates in appropriately chosen 3D gels or polymers can fuse to form tissue constructs of desired geometry. To demonstrate the feasibility, the teachings of the invention disclose an experimental realization of cell aggregate fusion (detail discussed in Example). In the experiment, cell aggregates of genetically transformed cells with controlled adhesive properties were employed and arranged to form a ring in gels of different chemical and mechanical properties. The results demonstrate that contiguous aggregates under appropriate conditions, defined by the composition of the embedding gel or polymers, indeed can fuse into structures of specified morphology.

[0022] The present invention further defines the Bio-ink particles as cell aggregates having the capacity 1) to be delivered by computer-aided jet-based or automatic cell dispenser-based deposition or "printing" and 2) to fuse into, or consolidate to form self-assembled histological constructs. Though Bio-ink particles may be in a

variety of sizes, the best mode is to pre-select the desired size of the aggregates. Controlling the size of aggregates is critical for their effective deposition using automated depositing devices. Aggregate size is for example limited by the capacity of nutrients to diffuse to the central cells and depends on cell type. The linear size of aggregates typically varies from about 100 micrometers to about 500 micrometers.

[0023] Bio-ink particles may vary in shape. The ideal shape of the aggregates is spherical or cylindrical to avoid the potential clogging of dispensers [210]. The Biophysical properties (e.g. cohesivity) of a particular type of Bio-ink particles depend on the nature of their constituent cells and the physical and chemical properties of the employed gels or polymers.

[0024] The composition of aggregates can be varied from homocellular (i.e. monocolor Bio-ink) to hybrid heterocellular (i.e. multicolor Bio-ink), and they can be fabricated to contain extracellular matrix in desired amount. Furthermore, cell aggregates can have elaborate internal structures with complex pre-built branching or interconnecting architecture.

[0025] The present invention also discloses the preparation of the Bio-ink particles. The Bio-ink particles may be fabricated by several methods. The classic "hanging drop" method is the simplest (cells in an inverted drop of tissue culture medium precipitate and aggregate). Shaking cell suspensions in appropriate laboratory flasks is another popular approach. Many modifications of these basic techniques may also be applied. Another effective method of making aggregates of controlled size is to centrifuge the suspension and then cut the resulting pellet into similar-sized fragments.

The cutting of pellet can be automated to produce identical cylinder-shaped pieces with an aspect, diameter to length, ratio of one. Subsequent incubation in a gyratory shaker, with its speed and duration of shaking appropriately chosen for each aggregate type, leads to the rounding of fragments into spheres.

[0026] According to the teachings of the invention, the gels or polymers employed in the dispensers need to be optimized in relation to the Bio-ink particles used in a specific application. Optimization of gel or polymers can be aided by modeling and computer simulation. A simple version of such a model considers the cells and gel volume elements as particles occupying the nodes of a discrete lattice. It assumes specific interactions between cells, cells and the gel and between gel volume elements. In this model it is only a certain combination of these three interaction parameters that eventually determines the evolution of the deposited structure (i.e. the fusion of the Bio-ink particles). Computer simulations of this model provide evolution patterns that match those obtained in applications with aggregates composed of living cells. The optimization of the gels or polymers for more complicated organ modules requires the generalization of this model.

[0027] To investigate shape changes of the evolving pattern, a simple three-dimensional model has been constructed, in which the sites of a cubic lattice are occupied either by point-like cells or gel volume elements. The total interaction energy, E of the system is written as

$$E = \sum_{<r,r'>} J(\sigma_r, \sigma_{r'}), \qquad (1)$$

where r and r' label lattice sites, and $< r, r' >$ signifies summation over neighboring sites, each pair counted once. First, second and third nearest neighbors are included, and it is assumed that a cell interacts with the same strength with all the 26 cells it comes into contact with (26 is the total number of first, second and third nearest neighbors of a given site in a cubic lattice). To specify occupancy, a spin value, σ, is assigned to each lattice site with values 0 for a "gel particle" and 1 for a cell. The interaction energy of two neighbors, $J(\sigma_r, \sigma_{r'})$, may take any of the values $J(0,0) = -\varepsilon_{gg}$, $J(1,1) = -\varepsilon_{cc}$ or $J(0,1) = J(1,0) = -\varepsilon_{cg}$. The positive parameters ε_{cc}, ε_{gg} and ε_{cg} account for contact interaction strengths for cell-cell, gel-gel and cell-gel pairs, respectively. More specifically, these are mechanical works needed to disrupt the corresponding bonds. (Note that ε_{cc} and ε_{gg} are works of cohesion, whereas ε_{cg} is work of adhesion per bond). The strength of cell-cell interaction may be determined experimentally either directly or by measuring the tissue surface tension. The cell-gel interaction is tunable by biochemical methods or by the concentration of the polymer forming the gel. The gel-gel "bond energy" is an effective measure of gel filament density, interaction and stiffness. It is determined by the specific chemistry of the gel.

[0028] The energy in Eq. 1 may be rewritten by separating interfacial and bulk terms in the sum. As a result we obtain

$$E = \gamma_{cg} B_{cg} + \text{const.} \qquad (2)$$

Here B_{cg} is the total number of cell-gel bonds, and $\gamma_{cg} = (\varepsilon_{cc} + \varepsilon_{gg})/2 - \varepsilon_{cg}$ is proportional to the cell-gel interfacial tension. The remaining terms in E do not change as the cellular pattern evolves. The inventive model is inspired by earlier efforts aiming at computer simulations of cell sorting, the morphogenetic phenomenon in which one of two, initially randomly intermixed cell populations sorts out and becomes surrounded by the other.

[0029] The evolution of the system is followed using Monte Carlo simulations, relying on a random number generator. The program identifies the cells on the aggregate-gel interface, picks one of them randomly, and exchanges it with an adjacent gel particle chosen by chance. The corresponding change in adhesive energy, ΔE, is calculated and the new configuration accepted with a probability $P = 1$ if $\Delta E \leq 0$ or $P = \exp(-\beta \Delta E)$ if $\Delta E > 0$. $\beta = 1/E_T$, is the inverse of the average biological fluctuation energy E_T, analogous to the thermal fluctuation energy, $k_B T$ (k_B-Boltzmann's constant, T - absolute temperature). In statistical mechanics this energy characterizes thermal agitation in a system of atoms or molecules. In the case of cells, it is a measure of the spontaneous, cytoskeleton driven motion of cells, able to break adhesive bonds between neighbors via membrane ruffling, or more generally, via membrane protrusive activity (e.g., filopodial extensions). By definition, a Monte Carlo step (MCS) or "unit of time," is completed when each cell in contact with the gel has been given the chance to move once. During each MCS the interfacial sites are selected in random order. The gel boundary is treated as a fixed physical limit of the system, and cells are constrained to move within the gel.

[0030] Some shapes correspond to local minima of the interaction energy. These represent metastable configurations. They are identified from plateaus in the plot of the total interaction energy vs. MCS, and are important for tissue engineering, for they can be made long-lived. This is illustrated in more detail in the simulation shown in Fig. 3A and Fig. 3B, where the initial state progresses towards a metastable toroidal configuration, whose energy is essentially unchanged in the entire interval between 10^4 and 6 x 10^4 MCS (Fig. 3A). Eventually the toroid becomes unstable, and at about 10^5 MCS it ruptures (Fig. 3B). Subsequent massive rearrangements lead to a pronounced energy decrease while the system evolves into three rounded aggregates. These remain stable for a long time because large spatial separations hinder their fusion into a single spheroid. Similar simulations showed that metastability depends on both system size and interaction strengths. Since the evolution of the cellular pattern is driven exclusively by energy minimization, and "time" is measured in MCS, in its present form the model cannot provide information on the true dynamical behavior of the system.

[0031] Once the structure reaches the metastable state, it can be stabilized by dissolving the supporting gel or polymer. In the simulations this corresponds to increasing the value of γ_{cg}/E_T. Indeed, if in the simulation shown in Fig. 3A this quantity is changed to $\gamma_{cg}/E_T = 2$ anywhere in the plateau region, the energy remains constant as long as the simulation is run.

[0032] Based on the inventive modeling method, the adhesive and mechanical properties of the embedding gels or polymers are critical for both cell aggregate fusion and the maintenance of the deposited construct. The ideal gels or polymers for organ

printing must allow cells or cell aggregates to survive and provide favorable conditions for post-processing self-assembly and tissue fusion. Several types of gels or polymers can fit the purpose: 1) thermo-reversible gels, 2) photo-sensitive gels, 3) pH-sensitive gels and 4) gels or polymers sensitive to specific molecular entities. The inventive modeling method is also capable of predicting cell aggregate "fusogenic" behavior and providing guidance in choosing and designing the optimal gels or polymers. The enclosed CD details the software / code enabling the inventive modeling method. The CD is hereby incorporated into this application.

[0033] Having described the invention, the following examples are given to illustrate specific applications of the invention including the best mode now known to perform the invention. These specific examples are not intended to limit the scope of the invention described in this application.

[0034] EXAMPLE

[0035] Experimental realization of three-dimensional structure with N-cadherin transfected Chinese Hamster Ovary (CHO) cell aggregates

[0036] To study the feasibility of engineering 3D tissue constructs of prescribed geometry, aggregates of living cells have been "manually printed" (i.e. embedded) into biocompatible gels. The experiments were performed with N-cadherin transfected CHO cells and gels or polymers with differing chemical composition.

[0037] **Cell Aggregate Preparation.** Chinese Hamster Ovary (CHO) cells, transfected with N-cadherin (courtesy of A. Bershadsky, Weizmann Institute, Rehovot, Israel), were infected with histone binding H2B-YFP retrovirus (kindly provided by R.D.

Lansford, Beckman Institute at California Institute of Technology). Confluent cell cultures (3-4x10^6 cells/75 cm^2 TC dish) grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL Grand Island, NY; supplemented with 10% FBS (US Biotechnologies, Parkerford, PA), 10μg/ml of penicillin, streptomycin, gentamicin, kanamycin, 400μg/ml geneticin), were washed twice with Hanks' Balanced Salt Solution (HBSS) containing 2mM $CaCl_2$, then treated for 10 minutes with trypsin 0.1% (diluted from 2.5% stock, Gibco BRL, Grand Island, NY). Depleted cells were centrifuged at 2500 RPM for 4 minutes. The resulting pellet was transferred into capillary micropipettes of 500 μm diameter and incubated at 37 C^0 with 5% CO_2 for 10 minutes. The firm cylinders of cells removed from the pipettes were cut into fragments (500 μm height), then incubated in 10-ml tissue culture flasks (Bellco Glass, Vineland, NJ) with 3 ml DMEM on a gyratory shaker at 120 RPM with 5% CO_2 at 37°C for 24-36 hours. This procedure provided spherical aggregates of similar size (~500 μm diameter), which has been reproduced in subsequent experiments.

[0038] **Cell Aggregate-Gel Structures.** NeuroGel™ (a biocompatible porous poly[N-(hydroxypropyl)methacrylamide] hydrogel) disks of 10 mm diameter and 2 mm thickness, containing RGD fragments (kindly provided by Stephane Woerly, Organogel Canada, Quebec) were washed three times with DMEM to eliminate the storage medium. A 0.5 mm wide, 0.5 mm deep circular groove was cut into a disk, then filled with 10 aggregates, placed contiguously to form a closed circle. The groove was refilled with the gel to completely embed the aggregates. This structure was incubated at 37°C, 5% CO_2 for 72 hours in a tissue culture dish containing 10 ml DMEM, washed with PBS, and

finally embedded in Tissue-Tek® OCT Compound (Electron Microscopy Sciences, Fort Washington, PA). The structure was slowly cooled (1 C^0/min) to –20 C^0 in a Nalgene freezing container (Nalgene Labware, Rochester, NY). To visualize aggregate fusion, at the end of the experiment cryosectioning was performed with a Reichert 2800N Frigocut cryotome (Reichert Jung, Arnsberg, Germany), yielding 10-16 μm thin slices mounted on microscope slides. Slices were examined on an Olympus IX-70 inverted microscope with fluorescent attachment at 4x magnification, as shown in Fig. 4.

[0039] To tune the strength of cell-gel interaction, further fusion experiments were conducted in rat-tail collagen type I (Sigma-Aldrich, St. Louis, MO). Collagen was dissolved in 1N acetic acid, and then treated with Ham's F12 medium with sodium bicarbonate. At room temperature this mixture gels in a few minutes depending on concentration. The gel-aggregate structure was achieved by creating a ring of ten aggregates on the top of a previously (almost) solidified collagen layer, then covering with liquid collagen that embedded the aggregates after gelation. These samples were incubated under the same conditions as described above. Working with 1.0, 1.2 and 1.7 mg/ml collagen, the samples were transparent, thus it was possible to follow pattern (i.e. toroid) evolution in time by phase contrast and fluorescent microscopy. Cell survivability was checked with Trypan Blue (Invitrogen, Carlsbad, CA) at the end of each fusion experiment. A minimal number of uniformly distributed dead cells were found.

[0040] **Experimental realization of three-dimensional structure.** The experiments were carried out by the system illustrated in Fig. 1 and utilized the inventive

Organ Printing method, in which N-cadherin transfected CHO cell aggregates were deposited or "printed" into gels. The experiments were also performed with fixed cell-cell adhesion and varying gel properties form 1.0, 1.2, and 1.7 mg/ml. As indicated by the teachings of the invention, the ability of aggregates to fuse depends on the mutual properties of the cell aggregates and gel or polymers, as expressed by the parameter γ_{cg}/E_T in the model. The transfected CHO cells' adhesive properties were quantitatively assessed, by measuring aggregate surface tension. The relative importance of cell-cell and cell-matrix interactions had also been investigated quantitatively.

[0041] The experimental results in Fig. 4 support the teachings. Fig. 4, shows initial (upper row) and final (lower row) cell aggregate configurations in the simulations and in experiments using various biocompatible gels. Panels A-B and K-L correspond to simulations with $\gamma_{cg}/E_T = 0.9$ and $\gamma_{cg}/E_T = 0.25$, respectively. The ten aggregates, each containing 925 cells are one cell diameter from each other in the starting configurations. The final configurations are reached after 25,000 and 50,000 MCS, respectively. Panels C-D, E-F, G-H and I-J correspond to experimental results, i.e, CHO cell aggregates embedded in a neurogel and in collagen gels of concentration 1.0, 1.2 and 1.7 mg/ml, respectively. The results in Fig. 4I-J show that collagen at concentration of 1.7 mg/ml is analogous to a permissive scaffold with small γ_{cg}/E_T. The results in Fig. 4C-D, E-F, and G-H show that eurogel and collagen at concentration of 1.0 and 1.2 mg/ml match more the definition of the non-permissive gel with high γ_{cg}/E_T. These gels favor much less (collagen), or not at all (neurogel) the dispersion of the cells into the scaffold, thus facilitating fusion.

[0042] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features herein before set forth and as follows in scope of the appended claims.

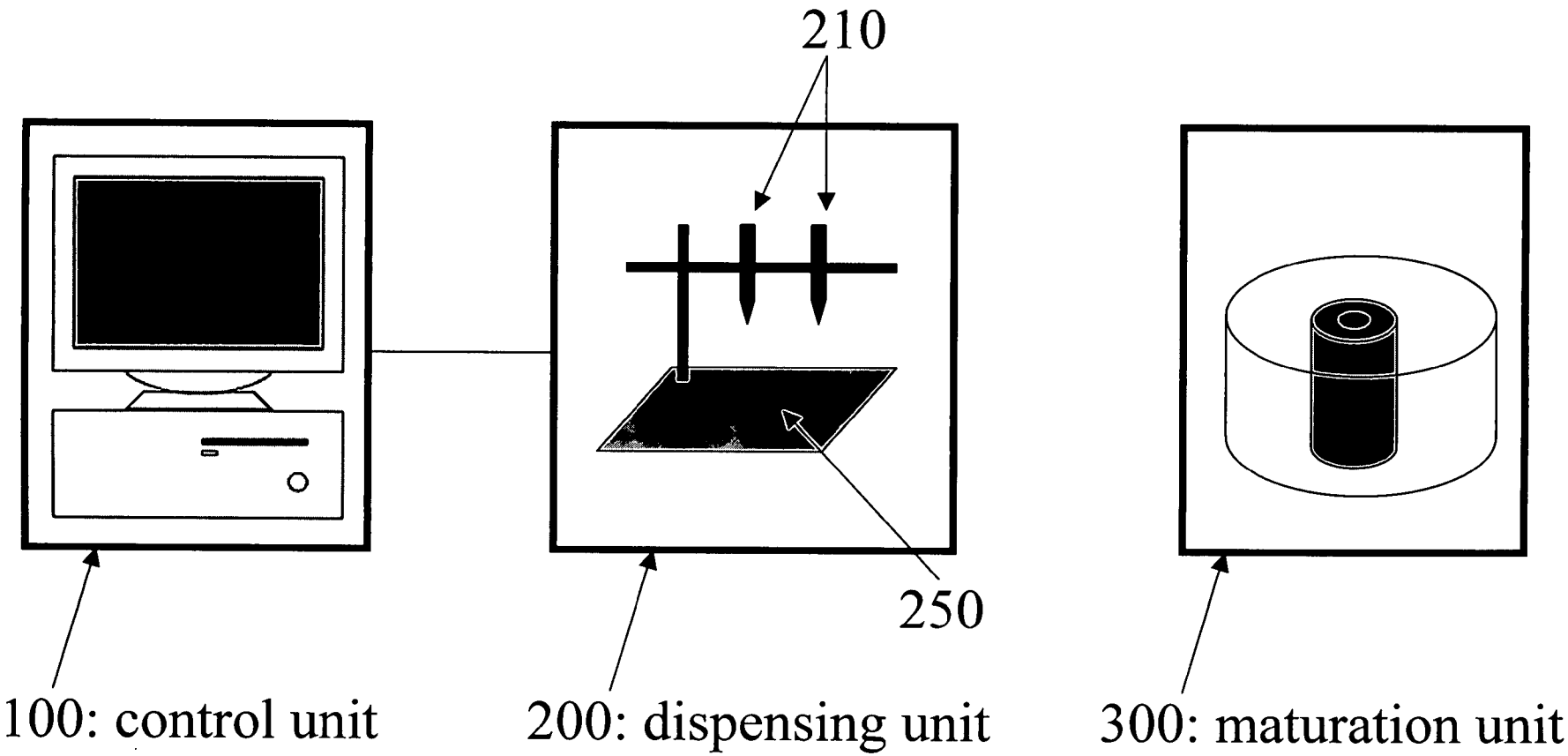

210
250
100: control unit
200: dispensing unit
300: maturation unit

Fig. 1

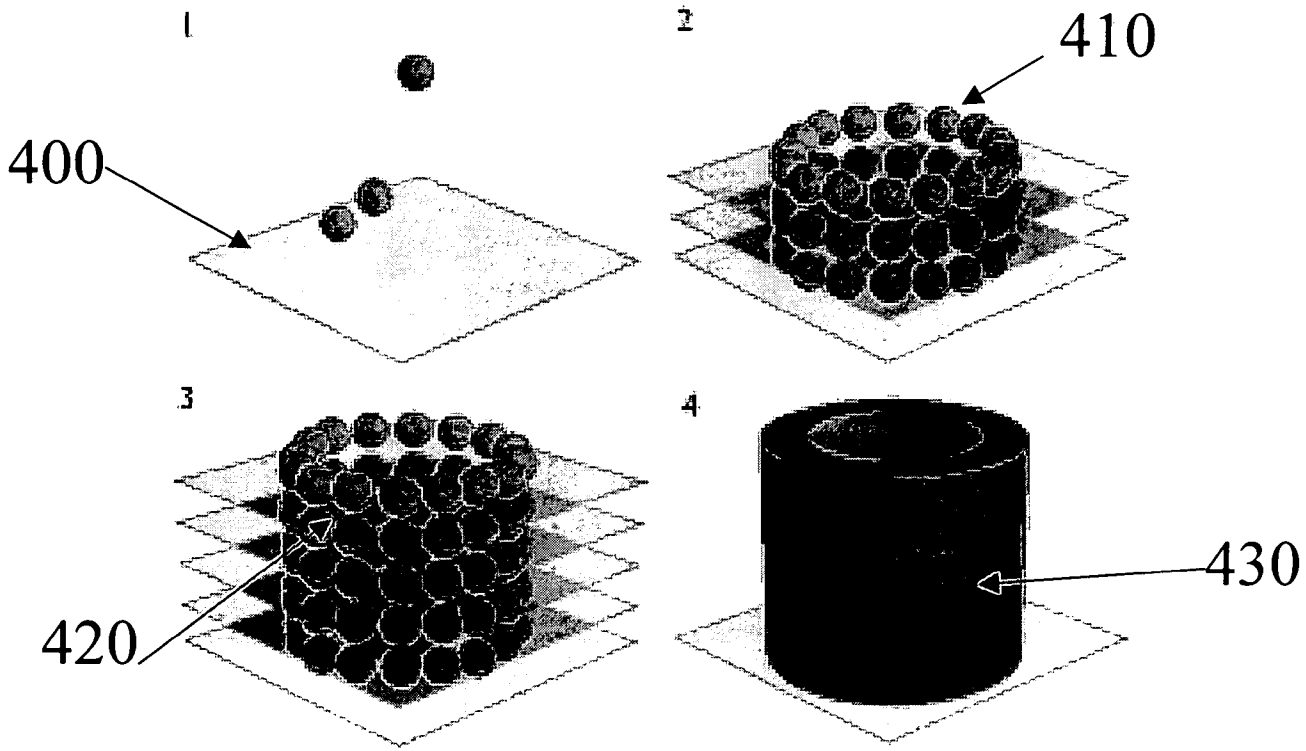
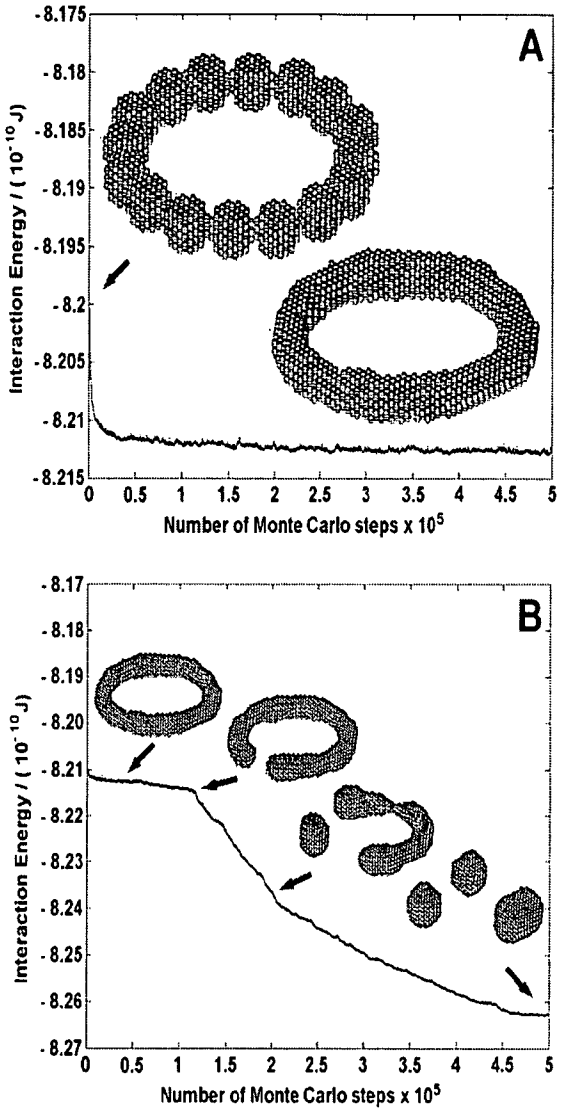


Fig. 2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B
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-8.21
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-8.24
-8.25
-8.26
-8.27
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

Fig. 3A & 3B

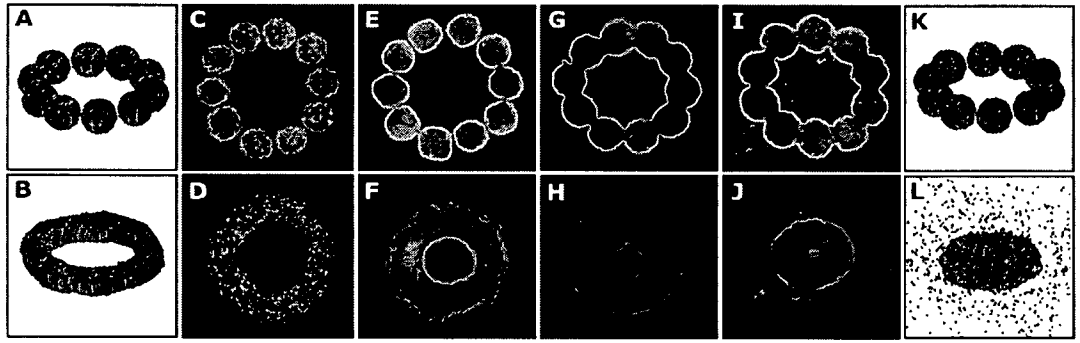

Fig. 4